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INVENTOR(s)/APPLICANT(s)

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TITLE OF THE INVENTION (280 characters max)

METHOD FOR CONDUCTING CHEMILUMINESCENT BINDING ASSAY

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Respectfully submitted,

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☐ Additional inventors are being named on separately numbered sheets attached hereto.

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Title

METHOD FOR CONDUCTING CHEMILUMINESCENT BINDING ASSAY

Field of the Invention

The present invention relates to a method for conducting a binding assay, and in particular to an immunoassay method that may be conducted on a Point Of Care (POC) device or an autoanalyzer.

Background to the Invention

The continuous needs to detect and quantify biomolecules (analytes) in various body fluids have resulted in the introduction of new and more accurate techniques that can be adapted for measuring a wide spectrum of different analytes. Most of these detection methods have been introduced into the clinical diagnostic field in recent years. Currently, a broad expansion in both the variety of analytes that may be readily and accurately determined as well as the methods for the determination have been witnessed. However, convenient, reliable, non-hazardous, highly sensitive and technically less challenging methods for detecting the presence of low concentrations of analytes in liquids is still desired, especially when the analyte may be present in body fluids in very low concentrations.

Several methods for the detection and quantification of substances of biological origin in fluid samples are currently employed. Bioanalytical assays, such as immunoassays and nucleic acid hybridization assays, which are based on binding between ligands and one or more members of specific binding pairs are widely used to determine the presence and quantity of analytes of interest, for example chemical constituents or substances

of a sample. In particular, immunoassays are widely employed detection and quantification methods in the clinical laboratory.

In a typical procedure of a sandwich immunoassay, an antibody against a particular antigen, known as a capture antibody, is immobilized to a solid surface. The sample under investigation is contacted with the solid surface under conditions that allow antigen in the sample to bind to the capture antibody. Another antibody known as a detection antibody is added. In the direct immunoassay format, the detector antibody is directly conjugated with a signal generating mechanism that allows the amount of the detection antibody to be quantified. In the indirect format, after the binding of the detection antibody to the antigen, another antibody against the detection antibody or another specific binding reaction that involves the detector antibody is utilized. This so-called anti-detector antibody is directly conjugated with a signal generating mechanism. The binding reaction and therefore the antigen level in the sample is quantified by quantifying the signal produced by the signal generating mechanism.

Several types of labeling material have been utilized for signal generation in the receptor-ligand binding assays. Radioactive atoms, such as ^{125}I, ^{131}I, ^{3}H and ^{14}C were commonly utilized as the label. Although radioactive labels for immunoassays are sensitive, they suffer commonly recognized disadvantages, including safety and the stringent regulatory requirements resulting in a relatively short reagent shelf life. Several alternative labeling methods are currently utilized in binding bioassays including colorimetric enzyme reactions, fluorescence and chemiluminescence

reactions. Enzymes commonly utilized as labels are horseradish peroxidase, alkaline phosphatase, B-galactosidase and glucose oxidase. Although enzymes have an advantage over radioactive labels in that they are very stable and need no special facilities and instrumentation, enzyme immunoassays are generally slower, laborious and less sensitive. Luminescent labels have been utilized as an alternative for radioactive or enzyme labels as they possess the ease of use advantage of radiolabels and the reagent stability advantage of enzymes. Fluorescence detection can be used with a much wider variety of enzymes. However, due to the difficulty of conventional fluorescence detection in discriminating between specific and nonspecific signals and therefore the practical assay detection limit, fluorescence assays lack the sensitivity of either radioactive and enzyme labels, making them seldom the assay method of choice for both research and clinical applications.

Chemiluminescent reactions as label of signal generation are the most sensitive and have been around for decades. Recent advances in DNA technologies have expanded the utilization of these labels as signal generators, but due to the limited number of known reactions which form chemiluminescent products, the luminescence assay method is currently under utilized. Also, due to the fact that luminescent reactions need one or more chemical activation steps, automation of these reactions is difficult, although needing less complex instrumentation than fluorescence. A large number of luminescence meters viz. luminometers, of various formats and sizes are available, although automation of luminescence is complicated and fully-automated

luminometers for carrying out binding assays are not available, at least in convenient, small-size analyzers.

The most common luminescence method utilized as a label for signal generation in binding assays is chemiluminescence, which is of several types, classified according to the method utilized for generating the luminescent signal viz. chemiluminescent and bioluminescent labels. Bioluminescence refers to the emission of light by biological molecules and utilizes bioluminescent proteins which can be true enzymes such as luciferases that catalyze the oxidation of luciferin with release of oxyluciferin and emitting light or photoproteins, that catalyze the oxidation of luciferin to emit light but do not release the oxidized substrate.

The calcium-sensitive photoproteins, including Aequorin, Obeln, Mnemiopsin, Berovin, Pholasin, Luciferases and photoproteins isolated from Pelagia, Cypridina and ostracods were widely researched and employed in binding assays. Furthermore, the genes of some of them have been cloned, permitting the production of large quantities. Aequorin is the most commonly studied and employed member of this group of calcium-sensitive photoproteins.

Native aequorin, abundant in jellyfish (Aequorea), has been purified and utilized as a label in varieties of monitoring systems. Native aequorin consists of a single polypeptide chain of MW 21,000 daltons (called apoaequorin), containing one mole each of tightly bound coelenterate luciferin and oxygen. This complex is stable in the absence of calcium ions. Aequorin can also be produced by recombinant DNA techniques, for example as discussed by Cormier, M. J., U.S. Patent 5,162,227 and Zenno. S. et al. in U.S. Patent 5,288,623. Furthermore,

modified forms of aequorin with enhanced bioluminescence properties have also been produced by recombinant DNA procedures as disclosed by Prasher, D. in U.S. Patent 5,360,728.

The mechanism of photon emission of aequorin is well understood. Aequorin has a high-affinity calcium ion and in the presence of elevated calcium ions, aequorin catalyzes the oxidation of luciferin to oxyluciferin in a single turnover event with the generation of a glow-type "flash reaction" which persists for approximately 10 seconds with a relatively high quantum yield. Although peak of light emission is initiated upon binding of three moles of calcium ions per mole of aequorin, binding of aequorin with trace of amount of free calcium results in partial oxidation of coelenterazine and yields apoaequorin, coelenteramide, CO and light.

As aequorin can be detected at the attomole level and the wavelength of its luminescence is very narrow and could be detected using commercially available luminometers, luminescence of aequorin offers many advantages including speed, high sensitivity and accuracy with a low background. Therefore, aequorin has proven useful as a label in binding assays. Furthermore, stable conjugates of aequorin with various binding reagents such as receptors, hormones, lectins, antibodies, antigens, DNA, RNA, oligonucleotides, and glycoproteins have been developed and a large number of such conjugates are commercially available.

When utilized in combination with streptavidin, biotinylated derivative of aequorin demonstrated the ability to detect nanogram to subnanogram amounts of the target analyte immobilized onto the wells of microtiter plates or nitrocellulose membranes, including proteins

and DNA. Marketed luminometers that employ aequorin are designed with injectors to inject calcium at a particular moment. Although several clinical testing assays that utilize aequorin have been recently introduced, the luminometers are not automated and tanks of solutions of calcium have to be included, which makes them awkward to utilize by non-specialized personnel. A luminescent specific binding assay using whole blood is disclosed by Pankratz et al in U.S. Patent 5,876,935.

In the cell of an organism, calcium (Ca) is an important intracellular second messenger for a wide variety of processes, which have physiological, biochemical and pathophysiological significance such as muscle contraction, neurotransmitter release, ion channel gating, exocytosis. Attempts to understand and measure the rapid changes and release of intracellular calcium have resulted into the introduction of a class of calcium-sensitive compounds called calcium-caging compounds. Calcium-caging compounds have the ability to be loaded with calcium and to unload their calcium upon stimulation. Unloading of the encased calcium may be induced by several methods, one of which is through exposure to light. Light-stimulation release of calcium from the caged compounds (called photolysis) is usually done by illumination for fractions of a second with laser pulses typically in the UV 350-400 nm region of the spectrum. Two different classes of Ca-caging compounds have been introduced; the BAPTA derivative such as the nitr-5 and nitr-7 and the EDTA or EGTA derivatives such as DM-nitrophen and nitrophenyl-EGTA. The latter class was designed to produce photosensitive derivatives of chelators with known high affinity for calcium, see US Patent 5,446,186 and U.S. Patent 4,981,985. The DM-

nitrophen and nitrophenyl-EGTA calcium-caging compounds offer the advantage of calcium-selectivity and upon irradiation, the chelated calcium cleaves with the subsequent cleaved remainders having a substantially lower affinity for the released calcium and therefore, large mounts of calcium are rapidly released. These photosensitive calcium-caging compounds are commercially available and have not been utilized beforehand in binding assays, whether immunoassays or nucleic acid binding assays.

Summary of the Invention

A method has now been found for conducting a receptor-ligand binding assay utilizing caged-calcium compounds and calcium-sensitive luminescent compounds.

Accordingly, one aspect of the present invention provides a method for conducting a binding assay to detect the presence of an analyte in a solution, comprising the steps of:

(a) contacting a first binding partner with said solution, said first binding partner being conjugated to a calcium-sensitive chemiluminescent material;

(b) after a further period of time, mobilizing the first binding partner in a predetermined direction along one side of an elongated matrix of a capture strip so as to contact the binding partner with a strip transversely located on said capture strip, said capture strip having immobilized second binding partner onto said transverse strip, said transverse strip additionally containing a calcium-caging compound,

(c) allowing a period of time sufficient for the binding partner to contact said second binding partner immobilized onto said transverse strip,

(d) exposing said transverse strip of said capture strip to a pulse of laser light to effect the release of calcium from the caged calcium compound; and

(e) measuring luminescence emitted by the calcium-sensitive luminescent material.

Another aspect of the present invention provides a method for conducting a binding assay to detect the presence of an analyte in a solution, comprising the steps of:

(a) contacting said solution with a first binding partner of a binding reaction, said first binding partner being immobilized on a solid surface, said solid surface being paramagnetic particles and said first binding partner being conjugated to calcium-sensitive luminescent material;

(b) after a period of time, mobilizing the paramagnetic particles in a predetermined direction along one side of an elongated matrix of a capture strip so as to contact the particles with a strip of a binding partner transversely located on said capture strip, said capture strip having the second binding partner immobilized onto said transverse strip, said transverse strip additionally containing a calcium-caging compound,

(c) allowing a period of time sufficient for the paramagnetic particles to contact said second binding partner immobilized onto said transverse strip,

(d) exposing said transverse strip of said capture strip to a pulse of laser light to effect the release of calcium from the caged calcium compound; and

(e) measuring luminescence emitted by the calcium-sensitive luminescent material.

In preferred embodiments of the invention, the method is an immunoassay for detecting and quantifying an

antigen, an immunoassay for detecting and quantifying an antibody, or a nucleic acid hybridization assay for detection and quantifying a particular sequence of nucleic acid.

In another embodiment, the solution is filtered prior to contacting the paramagnetic particles in step (a), especially filtered to remove calcium, the filter containing an agent for removal of calcium.

In another embodiment, the solution is whole blood, said whole blood being filtered prior to being contacted with the paramagnetic particles.

In a further embodiment, the luminescent material is calcium-sensitive luminescent material, especially aequorin, Obeln, Mnemiopsin, Berovin, Pholasin, Luciferases or photoproteins isolated from Pelagia, Cypridina and ostracods.

In still further embodiments, the laser emits a pulse of light in the range of 250-350 nm, and the luminescence is measured by a photomultiplier. In particular, the calcium-sensitive luminescent material is aequorin and photomultiplier detects light of 400-600 nm and is protected from the magnetic filed.

In other embodiments, the elongated capturing strip is formed of nitrocellulose or polyacrylamide and has a transverse section with immobilized second binding partner and impregnated with a caged calcium compound.

In a further embodiment, the calcium-caging compound is loaded with calcium. Preferably, the calcium-caging compound is nitro-5, nitro-7, DM-nitrophen or nitrophenyl-EGTA.

A further aspect of the invention provides a method for conducting a binding assay to detect the presence of an analyte in a solution, comprising the steps of:

(a) immobilizing a first binding partner of a binding reaction onto a solid surface, said solid surface being paramagnetic particles, said first binding partner being biotinylated;

(b) contacting the said first binding partner with said solution;

(c) contacting the solution with a second binding partner, said second binding partner being conjugated to a calcium-sensitive luminescent material;

(d) after a period of time, mobilizing the paramagnetic particles in a predetermined direction along one side of an elongated matrix of a capture strip so as to contact the particles with a strip transversely located on said capture strip, said capture strip having streptavidin immobilized onto said transverse strip, said transverse strip additionally contain a calcium-caging compound,

(e) allowing a period of time sufficient for the paramagnetic particles to contact said streptavidin immobilized onto said transverse strip,

(f) exposing said transverse strip of said capture strip to a pulse of laser light to effect the release of calcium from the caged calcium compound; and

(g) measuring luminescence emitted by the calcium-sensitive luminescent material.

In an embodiment, steps (b) and (c) are carried out simultaneously.

Yet another aspect of the invention provides a method for conducting a binding assay to detect the presence of an analyte in a solution, comprising the steps of:

(a) contacting a first binding partner with said solution, said first binding partner being biotinylated;

(b) after a period of time, contacting the solution with a second binding partner, said second binding partner being conjugated to a calcium-sensitive luminescent material;

(c) after a further period of time, mobilizing the binding partners in a predetermined direction along one side of an elongated matrix of a capture strip so as to contact the binding partners with a strip transversely located on said capture strip, said capture strip having streptavidin immobilized onto said transverse strip, said transverse strip additionally contain a calcium-caging compound,

(d) allowing a period of time sufficient for the binding partners to contact said streptavidin immobilized onto said transverse strip,

(e) exposing said transverse strip of said capture strip to a pulse of laser light to effect the release of calcium from the caged calcium compound; and

(f) measuring luminescence emitted by the calcium-sensitive luminescent material.

In an embodiment, steps (a) and (b) are carried out simultaneously.

In a further embodiment, the elongated capturing strip has a transverse section thereof impregnated with streptavidin and a calcium-caging compound.

In a still further aspect, the present invention provides an elongated capture strip for binding assays, said strip having a transverse section thereof impregnated with a binding partner and a caged calcium compound.

In preferred embodiments, the capture strip is formed from nitrocellulose or polyacrylamide.

In another embodiment, the capture strip is in a housing, especially within a support as a single use testing cartridge.

In a further embodiment, the binding partner is streptavidin.

A further embodiment of the invention provides a plastic cartridge comprising:

a housing with a receptacle for receipt of a sample, a reservoir containing biotinylated first binding partner immobilized onto paramagnetic particles and a second binding partner conjugated to calcium-sensitive chemiluminescence material, an elongated capturing strip within the housing and in fluid communication with the reservoir, said capture strip having a transverse section thereof impregnated with streptavidin and a calcium-caging compound, said transverse section being protected with a light barrier.

In a preferred embodiment, there is a filter between the receptacle and the reservoir, especially a filter containing an agent for removal of calcium.

A further embodiment provides apparatus for carrying out a binding assay comprising a housing enclosing (a) a receptacle to receive the plastic cartridge of Claim 28; (b) a means for removing the light protective layer over the transverse strip; (c) a magnet to provide a magnetic field; (e) a laser to project light on a pre-selected portion of the capture strip, and (f) a photomultiplier disposed to receive light emitted by the pre-selected portion of the capture strip.

Brief Description of the Drawings

The present invention is illustrated by the embodiment shown in the drawings, in which:

Fig. 1 is a schematic representation of a capture strip of the present invention;

Fig. 2 is a schematic representation of the method of the present invention; and

Fig. 3 is a schematic representation of apparatus of the present invention.

Detailed Description of the Invention

While the present invention may be used for detection and quantification of a binding partner of a binding reaction, it will be described herein with particular reference to a sandwich immunoassay for the detection and quantification of antigen that additionally employs a biotin-streptavidin reaction and paramagnetic particles, which is preferred.

Fig. 1 shows a capture strip, generally indicated by 1. Capture strip 1 has an elongated matrix 2. Elongated matrix 2 is formed from a matrix composition that will permit the paramagnetic particles with associated immune complex thereon to pass along the capture strip under the influence of a magnetic field. Examples of the matrix composition include nitrocellulose and polyacrylamide. Elongated matrix 2 has transverse strip 3 located towards one end, such end being opposed to inlet end 4. Transverse strip 3 contains both streptavidin and calcium-loaded calcium caged compounds or such other compounds as are disclosed herein.

Fig. 2 shows a plastic cartridge for carrying out the immunoassay reaction, generally indicated by 10. Plastic cartridge 10 has cartridge housing 11. Cartridge housing 11 has a sample receiving receptacle that contains a filter 12, a reservoir 13 for housing the paramagnetic particles 14 and the second binding partner

(Number),and particle path 15. Particle path 15 is in fluid communication with the reservoir 13 and leads from reservoir 13 into capture strip 16, where particle path 15 extends along elongated path 17 of capture strip 16 to transverse strip 18. Filter 12, reservoir 13 and capture strip 16 are all located within a holder 19 which forms part of plastic cartridge 10. It is to be understood that at least transverse strip 18 would have a peelable protective light barrier thereon which would be removed before use, i.e. before exposure to light from the laser. Additionally, the elongated capture strip is in communication with a discharge reservoir 20 at the opposite end of the sample receiving receptacle for receiving reagents that pass from the transverse strip.

Fig. 3 shows a testing platform apparatus, generally indicated by 30. Testing platform apparatus 30 has housing 31. Within the housing 31 are plastic cartridge 32, magnet 33, laser tip 34 and photomultiplier 35. Plastic cartridge 32 has been described previously, and could be accommodated within the receptacle of the housing 31 of the platform apparatus 30. Magnet 33 extends for the length of plastic cartridge 32. A tip of a laser 34 is directed at plastic cartridge 32 and, in particular, at transverse strip 18 of plastic cartridge 32, which has been described previously. Photomultiplier 35 is also directed at transverse strip 18.

Housing 31 additionally has display 36, which would typically be an LED display. Housing 31 would also contain appropriate controls and associated computer hardware and software to permit appropriate interpretation of the results obtained.

In use, a sample containing an antigen e.g. blood, is placed on filter 12. Liquid containing the target

analyte passes through filter 12 into reservoir 13, where it contacts the paramagnetic particles which has the first biotinylated binding partner (capture partner) immobilized onto it and the second binding partner conjugated to a calcium-sensitive luminescent label (detector partner). In addition, it is understood that when the cartridge is designed to detect and quantify an antigen, the first and second binding partners are antibodies. On the other hand, when the cartridge is designed for detecting an antibody, the first binding partner is an antigen while the second binding partner is an antibody.

The plastic cartridge 10 (32) is then placed in the testing platform 30 if it is not already located within the platform. It is understood that at least transverse strip 18 of capture strip 16 of plastic cartridge 10 would need to be protected from light. Such protection could be removed within testing platform 30, in a light-tight manner. Such removal could be automatic.

After allowing appropriate time for the binding reaction, the magnetic field is applied, using magnet 33. Then, the paramagnetic particles and attached immune complexes move along particle path 15 and into capture strip 16. The particles then pass along capture strip 16 until transverse strip 18 is reached. At that time, the particles become bound to streptavidin, already located in transverse strip 18, through the biotinylated binding partner immobilized onto the particles. Transverse strip 18 additionally contains a caged calcium compound.

After an appropriate time, which would depend in particular on the dimensions of the capture strip 16, but which conveniently could be 4-6 minutes, laser 34 is activated and sends a pulse of light onto transverse

strip 18. The light causes the release of calcium from the calcium-loaded caged calcium compound, which occurs essentially instantaneously. The calcium contacts the calcium-sensitive chemiluminescent material, which then glows for a short period of time in the range of 4-10 seconds. The light that is emitted is detected by the photomultiplier 35, and conveniently displayed on display 36.

Some examples of the source of the solution containing or suspected of containing the target analyte that is subjected to the method of the present invention are blood or blood products, saliva, or any other body fluids but other solutions could be tested.

Utilizing calcium-sensitive luminescent material as the signal generating label in binding assays requires that the solutions that will be contacting the calcium-sensitive luminescent conjugate have to be calcium-free before the moment of generating the light emission. Furthermore, when the goal is to determine the presence of an analyte in whole blood, the sample of blood normally must be pretreated to remove cellular components and hemoglobin, which can interfere with the specific signal of the binding assay. Filters impregnated in calcium-chelating agents would achieve both functions of removing the cellular components as well as calcium from the solutions that contain the target analyte.

The method of the invention disclosed herein utilizes any calcium-sensitive luminescent material for the signal generation in binding assays including, but not limited to, aequorin, mitrocomin, clytin, obelin, mnemiopsin, berovin, halistaurin and phialidin. In case of utilizing a calcium-sensitive luminescent photoprotein, other than aequorin, the optimal

wavelength, other than 490nm which is the optimal wavelength for detecting the aequorin signal, of the photomultiplier has to be adapted accordingly. For example, the wavelength may be 400-600 nm.

Although photolysis of the calcium caging compounds could be achieved by many light sources generating light within a wavelength from 250-350 nm, a laser source is convenient to accurately deliver light for less than 100 nanoseconds at a wavelength of 290nm. Upon the release of calcium from the caging compound when light-triggered and upon binding of three moles of calcium ions per mole of aequorin, the light emission is initiated with a flash of blue light that persists for approximately ten seconds. The generated light could then be measured with a suitable photomultiplier both as peak light or total photon counting.

Although native calicum-sensitive luminescent photoproteins are particularly useful as a label in the method of the present invention of carrying out binding assays, the availability of other modified, recombinant DNA-driven forms of these photoproteins with enhanced luminescence, due to either the ability of regeneration or a higher affinity for calcium, are also compatible with the method of the invention.

Although caging compounds such as liposomes has been extensively researched, the recent introduction of cation-specific caging compounds is particularly useful in carrying out the method of the invention. The recently introduced two classes of calcium-caging compounds which are derivative of chelating agents are particularly useful as they are more stable and the mechanism[s] of their triggering is well defined. In particular, the DM-nitrophen derivative of EDTA (ethylenedinitrilo

tetraacetic acid, disodium salt) and nitrophenyl-EGTA (ethylenebis(oxyethylenenitrilo) tetraacetic acid) have a very low affinity for calcium once light-triggered. Also, the wavelength of luminescence of the cleaved compounds is much different than that of the calcium-sensitive luminescent photoproteins, and these photosensitive calcium-caging compounds are commercially available.

Combining aequorin, which can be detected at the attomol level, together with exploiting the high affinity of biotin/streptavidin reaction offers a very high sensitivity of the method of the invention to measure analytes at a subnanogram level of detection. Furthermore, modified forms of streptavidin are also compatible with the method of the invention and both streptavidin and its derivatives could be easily immobilized onto the lateral transverse strip of the capture matrix strip.

According to the method of the invention for carrying out a binding assay, separation of the bound from free luminescent label is effected by applying a magnetic field. It will be recognized that the force on suspended magnetic particle subjected to a magnetic field urges the particle to move to stronger field regions, typically towards the pole of a magnet, and that the strength of the force depends both on the field gradient and magnetism induced in the particle by the field. Thus, for rapid separation, a strong separator and a highly magnetizable particle appear preferable.

Microscopic magnetic particles ranging from 0.7-1.5 microns are compatible with the method of the invention and may be used as they provide a large surface area for coating with proteins, for example, those disclosed in U.S. Patents 3,970,518; 4,018,886; 4,230,685; 4,267,234;

4,452,773; 4,554,088; and 4,659,678. However, smaller size paramagnetic particles of the size 0.03 to 10 micrometer as described in the US Patent 5,736,349 are more suitable as large size particles of magnetic material tend to adhere to one another after removal of the magnetic field, due to residual magnetism. Suitable magnetic materials include ferromagnetic, ferrimagnetic and superparamagnetic materials. Other suitable magnetic materials include oxides, such as, for example, ferrites, perovskites, chromites and magnetoplumbites. Nickel particles may also be used.

The magnetic separation apparatus/method used for separating of target analyte-bearing magnetic particles from test media will depend on the nature and size of the magnetic particle. The micron-size magnetic particles suitable in the invention are readily removed from solution by means of commercially available magnetic separation devices. These devices employ a single relatively inexpensive permanent magnet located external to a container holding the test medium. Examples of such magnetic separators are the MAIA Magnetic Separator manufactured by Serono Diagnostics, Norwell, Mass., the DYNAL MPC-1 manufactured by DYNAL, Inc., Great Neck, N.Y. and the BioMag Separator, manufactured by Advanced Magnetics, Inc., Cambridge, Mass.

In developing a bioassay, there are many considerations for the assay to attain value in the clinical laboratory. One consideration is the signal response to changes in the concentration of analyte. A second consideration is the ease with which the protocol for the assay may be carried out. A third consideration is the variation in interference from sample to sample. Also, ease of preparation and purification of the

reagents, availability of equipment, ease of automation and interaction with material of interest are some of the additional considerations in developing a useful assay.

The method of the invention for carrying out a binding assay offers improvement in such consideration. The invention offers the high sensitivity of luminescence, the availability, sensitivity and high quantum yield of calcium-sensitive luminescent material, particularly aequorin, the physical characteristic of calcium-sensitive luminescent material to response to changes in calcium without having to manually inject calcium, the availability of commercial luminometers with photomultipliers that could detect the generated photons without the interference of the magnetic field, and the development of solid chromatographic capturing matrices that offer the convenience of point of care testing. Most important, the large difference in the wave length of exciting the caged calcium (290 nm) and the wavelength of measuring the generated photons (450-500 nm) facilitates detection of emitted light without interference from the incident light from the laser.

The present invention of carrying receptor-ligand binding reaction utilizing a calcium-sensitive chemiluminescent label has been described herein with reference to the paramagnetic particle having the biotinylated first binding partner immobilized onto its surface, the chemiluminescent material conjugated to the second binding partner, and with the caged calcium compound being associated with the streptavidin to carry out a full sandwich immunoassay for detecting and quantifying an antigen as the preferred embodiment of the method invention. However, it is to be understood that the method of the invention is as equally beneficial in

detecting an antibody as well as a nucleic acid as the target analyte of a receptor-ligand binding reaction. Also, it is to be understood that the method of the invention could be carried out with the first binding partner conjugated to a calcium-sensitive luminescent material and immobilized onto paramagnetic particles and the second binding partner immobilized in the transverse strip of the capture strip together with the caged calcium compound.

CLAIMS:

1. A method for conducting a binding assay to detect the presence of an analyte in a solution, comprising the steps of:

(a) contacting a first binding partner with said solution, said first binding partner being conjugated to a calcium-sensitive chemiluminescent material;

(b) after a further period of time, mobilizing the first binding partner in a predetermined direction along one side of an elongated matrix of a capture strip so as to contact the binding partner with a strip transversely located on said capture strip, said capture strip having immobilized second binding partner onto said transverse strip, said transverse strip additionally containing a calcium-caging compound,

(c) allowing a period of time sufficient for the binding partner to contact said second binding partner immobilized onto said transverse strip,

(d) exposing said transverse strip of said capture strip to a pulse of laser light to effect the release of calcium from the caged calcium compound; and

(e) measuring luminescence emitted by the calcium-sensitive luminescent material.

2. A method for conducting a binding assay to detect the presence of an analyte in a solution, comprising the steps of:

(a) contacting said solution with a first binding partner of a binding reaction, said first binding partner being immobilized on a solid surface, said solid surface being paramagnetic particles and said first binding

partner being conjugated to calcium-sensitive luminescent material;

(b) after a period of time, mobilizing the paramagnetic particles in a predetermined direction along one side of an elongated matrix of a capture strip so as to contact the particles with a strip of a binding partner transversely located on said capture strip, said capture strip having the second binding partner immobilized onto said transverse strip, said transverse strip additionally containing a calcium-caging compound,

(c) allowing a period of time sufficient for the paramagnetic particles to contact said second binding partner immobilized onto said transverse strip,

(d) exposing said transverse strip of said capture strip to a pulse of laser light to effect the release of calcium from the caged calcium compound; and

(e) measuring luminescence emitted by the calcium-sensitive luminescent material.

3. The method of Claim 2 which is an immunoassay for detecting and quantifying an antigen.

4. The method of Claim 2 which is an immunoassay for detecting and quantifying an antibody.

5. The method of Claim 2 in which the binding assay is nucleic acid hybridization assay for detection and quantifying a particular sequence of nucleic acid.

6. The method of Claim 2 in which the solution is filtered prior to contacting the paramagnetic particles in step (a).

7. The method of Claim 2 in which the solution is whole blood, said whole blood being filtered prior to being contacted with the paramagnetic particles.

8. The method of Claim 2 in which the solution is filtered to remove calcium, said filter contains an agent for removal of calcium.

9. The method of Claim 2 in which the luminescent material is calcium-sensitive luminescent material.

10. The method of Claim 2 in which the calcium-sensitive luminescent material is aequorin.

11. The method of Claim 2 in which the calcium-sensitive luminescent material is selected from Obeln, Mnemiopsin, Berovin, Pholasin, Luciferases and photoproteins isolated from Pelagia, Cypridina and ostracods.

12. The method of Claim 2 in which the laser emits a pulse of light in the range of 250-350 nm.

13. The method of Claim 2 in which the luminescence is measured by a photomultiplier.

14. The method of Claim 2 in which the calcium-sensitive luminescent material is aequorin and photomultiplier detects light of 400-600 nm and is protected from the magnetic filed.

15. The method of Claim 2 in which the elongated capturing strip is formed of nitrocellulose or

polyacrylamide and has a transverse section with immobilized second binding partner and impregnated with a caged calcium compound.

16. The method of Claim 2 in which the calcium-caging compound is loaded with calcium.

17. The method of Claim 2 in which the calcium-caging compound is nitro-5, nitro-7, DM-nitrophen or nitrophenyl-EGTA.

18. A method for conducting a binding assay to detect the presence of an analyte in a solution, comprising the steps of:

(a) immobilizing a first binding partner of a binding reaction onto a solid surface, said solid surface being paramagnetic particles, said first binding partner being biotinylated;

(b) contacting the said first binding partner with said solution;

(c) contacting the solution with a second binding partner, said second binding partner being conjugated to a calcium-sensitive luminescent material;

(d) after a period of time, mobilizing the paramagnetic particles in a predetermined direction along one side of an elongated matrix of a capture strip so as to contact the particles with a strip transversely located on said capture strip, said capture strip having streptavidin immobilized onto said transverse strip, said transverse strip additionally contain a calcium-caging compound,

(e) allowing a period of time sufficient for the paramagnetic particles to contact said streptavidin immobilized onto said transverse strip,

(f) exposing said transverse strip of said capture strip to a pulse of laser light to effect the release of calcium from the caged calcium compound; and

(g) measuring luminescence emitted by the calcium-sensitive luminescent material.

19. A method for conducting a binding assay to detect the presence of an analyte in a solution, comprising the steps of:

(a) contacting a first binding partner with said solution, said first binding partner being biotinylated;

(b) after a period of time, contacting the solution with a second binding partner, said second binding partner being conjugated to a calcium-sensitive luminescent material;

(c) after a further period of time, mobilizing the binding partners in a predetermined direction along one side of an elongated matrix of a capture strip so as to contact the binding partners with a strip transversely located on said capture strip, said capture strip having streptavidin immobilized onto said transverse strip, said transverse strip additionally contain a calcium-caging compound,

(d) allowing a period of time sufficient for the binding partners to contact said streptavidin immobilized onto said transverse strip,

(e) exposing said transverse strip of said capture strip to a pulse of laser light to effect the release of calcium from the caged calcium compound; and

(f) measuring luminescence emitted by the calcium-sensitive luminescent material.

20. The method of Claim 18 in which, steps (b) and (c) are carried out simultaneously.

21. The method of Claim 19 in which, steps (a) and (b) are carried out simultaneously.

22. The method of Claim 18 in which the elongated capturing strip has a transverse section thereof impregnated with streptavidin and a calcium-caging compound.

23. An elongated capture strip for binding assays, said strip having a transverse section thereof impregnated with a binding partner and a caged calcium compound.

24. The capture strip of Claim 23 in which the capture strip is formed from nitrocellulose or polyacrylamide.

25. The capture strip of Claim 23 in which the capture strip is in a housing.

26. The capture strip of Claim 23 in which the capture strip is housed within a support as a single use testing cartridge.

27. The capture strip of Claim 23 in which the binding partner is streptavidin.

28. A plastic cartridge comprising:

a housing with a receptacle for receipt of a sample, a reservoir containing biotinylated first binding partner immobilized onto paramagnetic particles and a second binding partner conjugated to calcium-sensitive chemiluminescence material, an elongated capturing strip within the housing and in fluid communication with the reservoir, said capture strip having a transverse section thereof impregnated with streptavidin and a calcium-caging compound, said transverse section being protected with a light barrier.

29. The plastic cartridge of Claim 28 in which there is a filter between the receptacle and the reservoir.

30. The plastic cartridge of Claim 28 in which the filter contains an agent for removal of calcium.

31. Apparatus for carrying out a binding assay comprising a housing enclosing (a) a receptacle to receive the plastic cartridge of Claim 28; (b) a means for removing the light protective layer over the transverse strip; (c) a magnet to provide a magnetic field; (e) a laser to project light on a pre-selected portion of the capture strip, and (f) a photomultiplier disposed to receive light emitted by the pre-selected portion of the capture strip.

ABSTRACT OF THE DISCLOSURE

A method for conducting a receptor-ligand binding reaction of a solution containing or suspected of containing the target analyte. The method comprises the steps of bonding the first binding partner to the surface of a paramagnetic particle, conjugating a second binding partner to a calcium-sensitive luminescent compound; contacting the first and second binding partners with the solution to be tested, immobilizing the paramagnetic particles along a capture strip that has a transverse strip containing streptavidin and containing a caged calcium compound, exposing the transverse strip to a pulse of laser light to effect the release of calcium from the caged calcium compound, and measuring luminescence emitted by the calcium-sensitive luminescent material. The method may be used in the testing of blood. An apparatus is also disclosed.

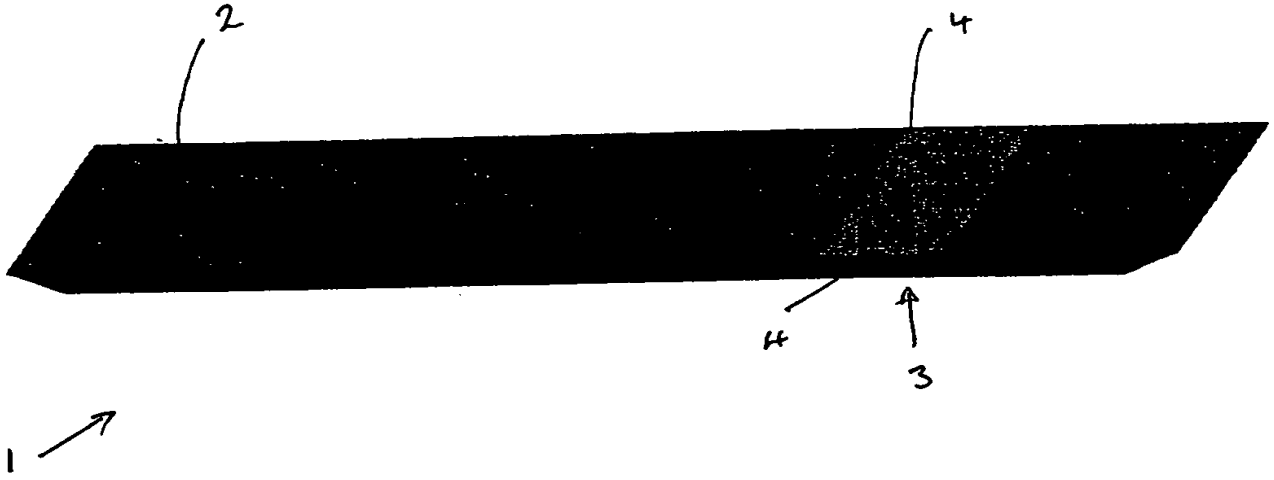

Fig. 1

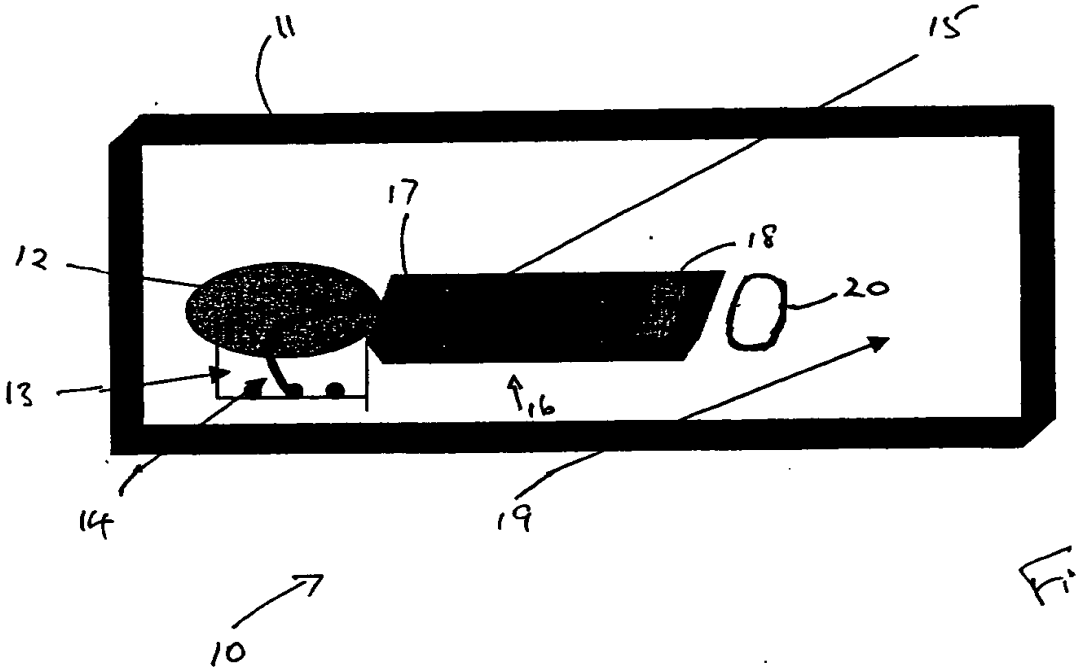

Fig. 2

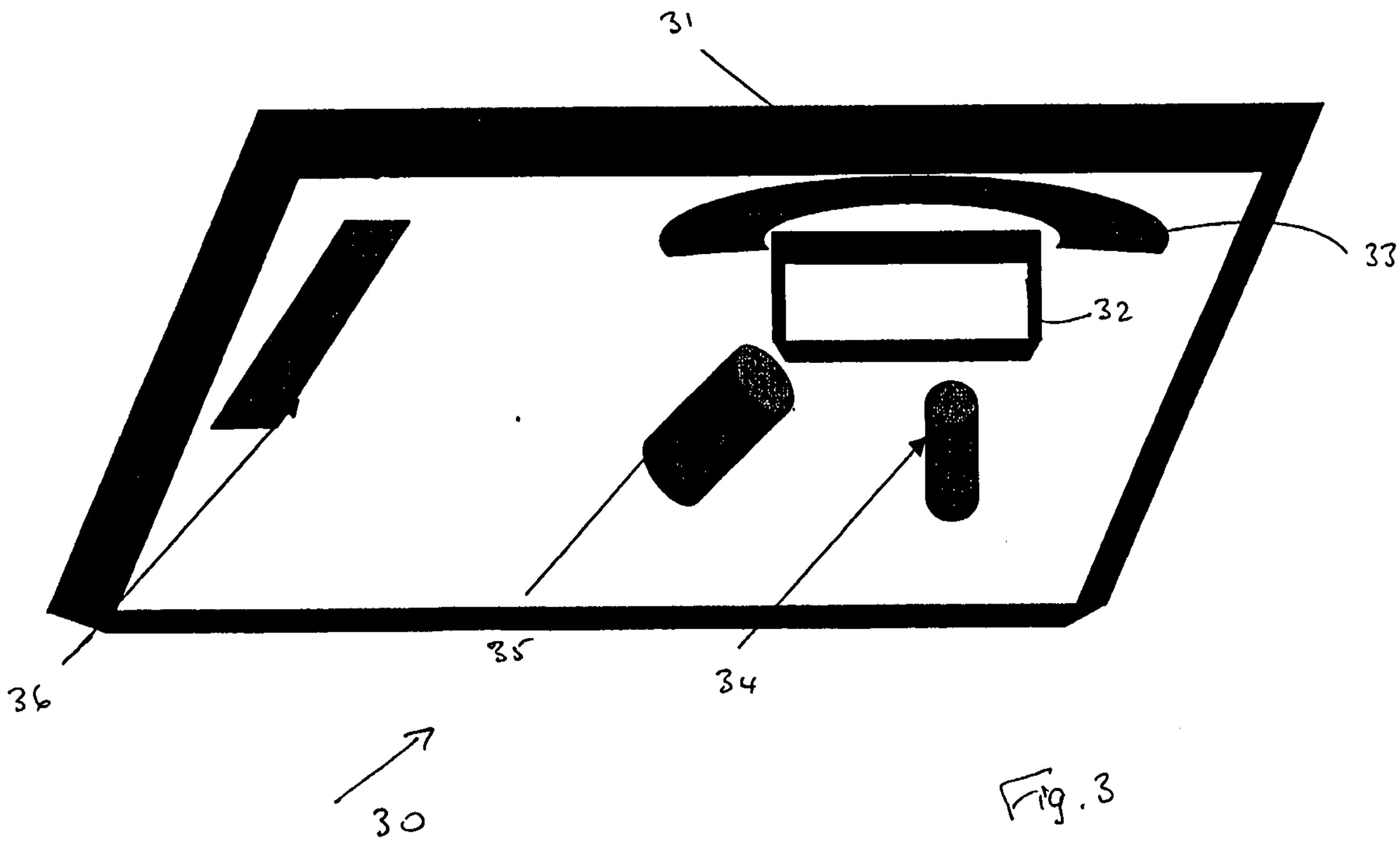

Fig. 3